WO 03/103378 PCT/US03/18393

Claims:

10

- 1. A method of making a diploid transgenic fish comprising:
- (a) introducing an exogenous nucleic acid sequence into the genome of a cultured cell derived from a progenitor fish;
 - (b) transplanting the nucleus of the cell of step (a) into an enucleated egg derived from a parental fish; and
 - (c) placing the egg generated by step (b) into conditions suitable for embryonic fish development so that a diploid transgenic fish is made.

gene product expressed therein that is encoded by the exogenous nucleic acid sequence.

2. The method of claim 1, wherein the transgenic fish has at least one exogenous

- 3. The method of claim 1, wherein the transgenic fish has at least one endogenous gene product that is inactivated by the exogenous nucleic acid sequence.
 - 4. The method of claim 1, wherein the exogenous nucleic acid comprises a promoter element or an enhancer element.
- 20 5. The method of claim 1, wherein the transgenic fish is fertile.
 - 6. The method of claim 1, wherein the cultured cell is a fibroblast derived from the embryo of the progenitor fish.
- 7. The method of claim 6, wherein the embryonic fibroblast cell derived from the progenitor fish has been maintained in cell culture for an amount of time sufficient for at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170 or 180 cell divisions prior to transplanting the nucleus of the embryonic fibroblast into an enucleated egg.

WO 03/103378 PCT/US03/18393

8. The method of claim 1, wherein the cultured cell derived from the progenitor fish has been frozen prior to step (a) or step (b).

- 9. The method of claim 1, wherein the progenitor fish are of the species Danio rerio, Oryzias latipes, Misgurnus anguillicaudatus, Salmo irdeus, Salmo salar, Oreochromis nilotica, Parasilurus asoltus, Mylopharyngodon poceus, Ctnopharyngodon idellus, Hypophthalmichihys molivrix, Aristichthys nobilis, Cyprinus Carpio or Carassius aurantus.
- 10. A transgenic fish made according to the method of claim 1.
 - 11. A method of making a progeny fish comprising:
 - (a) obtaining a cell from a progenitor fish,

15

- (b) maintaining the cell in in vitro culture,
- (c) transplanting the nucleus of the cell of step (b) into an enucleated egg from a parental fish; and
- (d) placing the egg generated by step (c) into conditions suitable for embryonic 20 fish development so that the progeny fish is made, wherein the progeny fish is fertile.
 - 12. The method of claim 11, wherein the progeny fish is diploid.
- 25 13. The method of claim 11, wherein the progeny fish is a transgenic fish.
 - 14. The method of claim 13, wherein the transgenic fish expresses at least one exogenous gene product encoded by the transgene.
- 30 15. The method of claim 13, wherein the transgenic fish has at least one endogenous gene product that is inactivated by the transgene.

WO 03/103378 PCT/US03/18393

16. The method of claim 13, wherein the transgenic fish comprises an exogenously introduced promoter element or enhancer element.

- 17. The method of claim 11, wherein the cell is maintained in in vitro culture an amount of time sufficient to:
 - (i) introduce an exogenous nucleic acid sequence into the genome of the cell; and
 - (ii) identify the cell containing the an exogenous nucleic acid sequence within a plurality of cells comprising the cell having the exogenous nucleic acid sequence and a cell lacking the exogenous nucleic acid sequence.

10

- 18. The method of claim 11, wherein the cell is maintained in culture an amount of time sufficient for at least 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170 or 180 cell divisions.
- 15 19. The method of claim 11, wherein the cell is an embryonic fibroblast derived from an embryo of the progenitor fish.
- 20. The method of claim 11, wherein the progenitor fish are of the species Danio rerio, Oryzias latipes, Misgurnus anguillicaudatus, Salmo irdeus, Salmo salar,
 20 Oreochromis nilotica, Parasilurus asoltus, Mylopharyngodon poceus, Ctnopharyngodon idellus, Hypophthalmichihys molivrix, Aristichthys nobilis, Cyprinus Carpio or Carassius aurantus.
 - 21. A progeny fish made according to the method of claim 11.

25